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Microgeographic population structure of green swordail fish: genetic differentiation despite abundant migration

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Abstract

Swordtails (*Xiphophorus*; Poeciliidae) have figured prominently in research on fish mating behaviours, sexual selection, and carcinogenesis, but their population structures and dispersal patterns have been relatively neglected. Using nine microsatellite loci, we estimated genetic differentiation in *Xiphophorus helleri* within and between adjacent streams in Belize. The genetic data were complemented by a tagging study of movement within one stream. In the absence of physical dispersal barriers (waterfalls), population structure followed an isolation by distance (IBD) pattern. Genetic differentiation (F_{ST} up to 0.07) was significant between and within creeks, despite high dispersal in the latter as judged by the tagging data. Such heterogeneity apparently was a result of genetic drift in local demes, due to small population sizes and highly skewed paternity. The IBD pattern was interrupted by waterfalls, boosting F_{ST} above 0.30 between adjacent samples across these barriers. Overall, our results are helpful in understanding the interplay of evolutionary forces and population dynamics in a small fish living in a changeable habitat.

Keywords: gene flow, genetic drift, microsatellites, physical barrier, tagging, *Xiphophorus helleri*

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Introduction

Subdivision of a species into genetically distinct populations is a common feature of the biological world (Avise 2000, 2004; Beaumont & Hoare 2003; Palumbi 2004). If measured by neutral genetic markers such as microsatellites (Goldstein & Schlötterer 1999), the inferred current population structure is a result of historical interactions between the homogenizing effect of migration and the differentiating influence of genetic drift (Beaumont & Hoare 2003; Knopp & Merilä 2009). Establishing the smallest scale at which genetic differentiation occurs is important for understanding such interactions. For example, significant neutral genetic differentiation in the face of apparent high dispersal might indicate the presence of hidden reproductive isolation (Knowlton 2000) or be a result of large stochastic variability in reproductive success (Hedgecock 1994a,b). Microsatellites are especially useful for studying microgeographic variation because they tend to be highly variable and can discern even small genetic differences (Estoup *et al.* 1998; but see Waples 1998). Evidence for genetic differentiation on fine geographical scales has been reported in a number of fishes (Avise & Felley 1979; White & Turner 1984; Smith *et al.* 1989; Congdon 1995; Crispo *et al.* 2006), with some studies making a special effort to distinguish between spatial and temporal variation (Garant *et al.* 2000; Hansen *et al.* 2002; Jensen *et al.* 2005b; Fraser *et al.* 2007). Although estimates of gene flow often are obtained through the use of genetic markers, their use in conjunction with more direct observational methods (such as tagging studies) can offer additional insights (Slatkin 1987; Wilson *et al.* 2004).

Species of *Xiphophorus* (platyfish and swordtails) have been the subject of many studies in genetics, systematics, biogeography, oncology and mating behaviour (reviewed in Meffe & Snelson 1989; Meyer *et al.* 1994; Kallman & Kazianis 2006; Meierjohann & Schartl 2006).

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However, basic data on population structure, gene flow, and dispersal in members of this genus remain sparse (Lucas & Baras 2001, p. 204; but see Gutierrez-Rodriguez *et al.* 2007, 2008).

In nature, male green swordtails form dominance hierarchies (Franck & Ribowski 1993). Such systems may promote sex-biased dispersal if subordinate males move to other sites in search of mating partners. Field observations also suggest that subordinates can become satellites of the dominant males, constantly attempting to intrude into their home ranges (Franck *et al.* 1998). It is also possible that females are the more dispersive sex as they seem indifferent to holding home ranges and thus may be freer to roam (Franck *et al.* 1998). Gender-biased dispersal potentially causes differences in population structure between the sexes, with the more dispersive sex expected to be less genetically structured and to display larger heterozygote deficits (Goudet *et al.* 2002). Signatures of sex-biased dispersal on population structure can be tested with bi-parentally inherited molecular markers, although several assumptions about species' biology should be fulfilled for such tests to be effective (Goudet *et al.* 2002; Prugnolle & de Meeus 2002). Despite such potential difficulties with genetic methods, several studies have successfully detected dispersal differences between the sexes: e.g., in cichlids (Knight *et al.* 1999; Taylor *et al.* 2003a), three-spined sticklebacks (Cano *et al.* 2008), and salmonids (Bekkevold *et al.* 2004; Fraser *et al.* 2004).

The native distribution of the green swordtail (*X. helleri*) stretches for a thousand kilometers along the Atlantic slope of Middle America, from northeastern Guatemala and northern Honduras to southeastern Veracruz, Mexico (Miller 2005; Kallman & Kazianis 2006). Habitats for the species range from coastal plain rivers to mountain streams as high as 1500 m (Kallman & Kazianis 2006). Many distinctive pigment patterns are known, and their localized distributions suggest a complex population structure. *X. helleri* has also been introduced into other parts of the world (Couternay & Meffe 1989) by fish hobbyists.

Green swordtails in our study area occupy creeks and streams running through a complex landscape that can make dispersal difficult. Some local populations occupy small pools that can be isolated or even decimated during dry seasons. Such pools typically are inhabited by only a few adults (perhaps several dozen at most), suggesting that genetic drift may be a major force in shaping population genetic structure. Furthermore, sexual selection is thought to play a significant role in swordtails (Tatarenkov *et al.* 2008), and this can additionally distort allele frequency dynamics due to large variances across males in progeny production (Ritchie *et al.* 2007). However, sufficiently high migration should counteract the stochastic drift of allele frequencies, and perhaps generate the appearance of panmixia over local scales yet isolation by distance patterns over larger areas.

The purpose of this study was to investigate genetic structure of green swordtail populations at small (tens of meters) to medium (several kilometers) scales. Specifically, we wanted to determine the smallest spatial scale at which differentiation is discernible, and identify the factors responsible. Results of our genetic survey were complemented by direct observations on dispersal based on physical tagging. Specifically, we addressed the following: (a) What is the extent of movement in a creek as judged by the dispersal of marked individuals? (b) Does genetic differentiation occur within sections of creeks lacking obvious barriers to movement? (c) How concordant are the tagging and genetic results? (d) Is there evidence for sex-biased dispersal? (e) What is the genetic structure of populations separated by waterfalls? (f) Does gene flow occur across waterfalls, and if so is it downstream only? (g) How does genetic variation within creeks compare to that between creeks? and (h) Is there evidence for isolation by distance?

Materials and methods

Environments and samples

Our study focused on creeks entering a section of Bladen Branch River in the foothills of the Maya Mountains in Belize, which contain the green swordtails as the only member of the genus *Xiphophorus*. Water-flow in these creeks is continuous during the wet season (June–December), but shallow sections dry out and deeper pools often become physically separated with no surface flow between pools during the dry season. Green swordtails survive the dry season in these quasi-discrete pools (typically a few to several dozen adults per pool), but some pools with fish desiccate completely by the end of the dry season in some years. It is worth noting that pools in one of the creeks we studied, Firetail Creek, remained connected during the dry season study period, albeit only by small trickles of water. These creeks run through mountainous terrain and are further subdivided by waterfalls that appear to be formidable obstacles to upstream dispersal. Nonetheless, in one of the four creeks we examined, Firetail Creek, green swordtails inhabited the area above waterfalls. These upper parts of creeks have uninterrupted water-flow throughout the year.

Tissue samples for genetic assay were collected in December, 2006, and April-May, 2007, from pools in each of the four creeks (Oro, O; Richardson, R; Firetail, FT; First, C1) running into Bladen Branch River in

southeastern Belize (Fig. 1). One additional site (A) was an offshoot of the main river, separated from it by a dry gravel bed. The most distant creeks (Oro and First) were separated by 16 km of river, and the waterway distances between particular collection sites ranged from 50 m (between FT12 and FT13) to 21 km (between O1 and U4). Firetail Creek was chosen for the most detailed microgeographic analysis, with nine sites collected along the downstream portion of the Creek (below the first waterfall, which was approximately seven meters high) plus three sites from the upstream (above the waterfall) section that we will henceforth refer to as U sites. These three U sites were also separated from one another by smaller (2–4 m high) waterfalls. Each collection site in a creek corresponded to a pool several meters long, separated from other pools by gravel beds. In addition to the sites already mentioned, we also collected and genotyped 27 fish from several pools in lower Firetail Creek where sample sizes were too small for population genetic analysis, but we included these fish in our movement analyses based on physical tagging.

Tissue samples were collected as caudal fin clips preserved in DMSO solution (Seutin *et al.* 1991) from individuals that were usually released immediately afterwards or kept as part of another study (Tatarenkov *et al.* 2008).

Tagging

The original purpose of tagging was to ensure exhaustive genetic sampling of potential sires in our parentage study (Tatarenkov *et al.* 2008). However, these data can also be used to study dispersal. We caught males throughout the lower part of Firetail Creek at the beginning (December), and end (April–May) of the dry season in 2006/2007. Upon capture in December, males were anesthetized, marked individually with coloured, biocompatible elastomer tags (Northwest Marine Technology, Inc.), clipped for a caudal fin sample, and released 1 day later at the same site. If a tagged fish was observed or captured later, its presence at the old or new site was recorded.

Microsatellite genotyping

DNA extraction, PCR amplification, and genotyping at nine microsatellite loci were accomplished by procedures described in Tatarenkov *et al.* (2008).

Statistical analyses

Allelic richness and basic statistics were calculated in FSTAT (version 2.9.3.2; Goudet 1995). Departures from Hardy–Weinberg equilibrium toward heterozygote deficiency (Rousset & Raymond 1995) were assessed separately for each locus using exact tests as implemented in Genepop (version 4.0; Rousset 2008). Significance of F_{IS} across loci and populations, as well as the significance of linkage disequilibrium for each pair of loci in each population, were likewise obtained with exact tests in Genepop. To avoid type I errors, we performed sequential corrections for multiple tests using Dunn-Sidak's method (Rice 1989; Sokal & Rohlf 1995, p. 239–

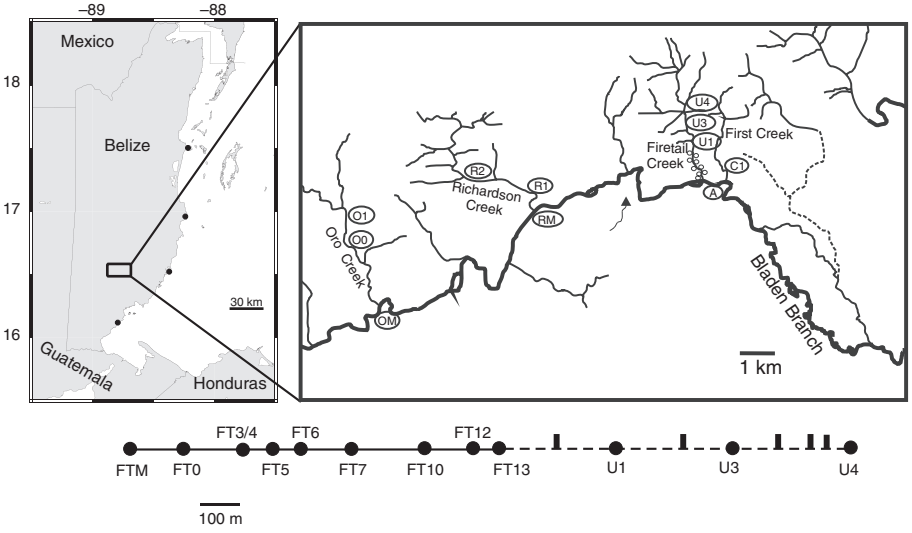

Fig. 1 Sample locations (sites are labelled as in Table 1). In the schematic arrangement of sample sites at the bottom of the figure, the continuous line indicates the downstream (below the waterfall) portion of Firetail Creek, and the broken line represents the upstream (above the waterfall) section. Waterfalls (two to seven meters high) in the upstream section of Firetail Creek are shown as vertical bars.

242). The presence of null alleles at suspect loci was further checked with MICRO-CHECKER (van Oosterhout *et al.* 2004).

Genic differentiation at each locus among populations was evaluated with exact tests in Genepop. Significance of population differentiation over all loci was determined with Fisher's test of combined probabilities (Sokal & Rohlf 1995, p. 794). Pairwise F_{ST} values were calculated and their significance was assessed by randomizing multi-locus genotypes in the program FSTAT (Goudet 1995), with appropriate Bonferroni correction. The association between pairwise genetic differentiation (F_{ST}) and waterway distances between collection sites was evaluated with Mantel tests as implemented in the program Genepop. Waterway distances were estimated from maps, or measured directly (for the sites in lower Firetail Creek).

The partial Mantel test (Bohonak 2002) was used to evaluate the structuring roles of both waterfalls and geographical (waterway) distances. Three tests were conducted: (a) accounting only for the largest waterfall, so that the U sites were not considered separated from each other by waterfalls; (b) considering only waterfall presence/absence between sites, without taking into account the number of waterfalls; and (c) accounting for the number of waterfalls (>2 m high) between sites. All tests were conducted using the program IBDWS (version 3.16; Bohonak 2002; Jensen *et al.* 2005a).

Differences in dispersal pattern between the sexes are expected to affect both intra- and interpopulation genetic parameters (e.g. Goudet *et al.* 2002). For example, F_{ST} and the assignment index are expected to be lower, whereas F_{IS}, expected heterozygosity, and the variance of the assignment index should be higher in the more dispersing sex (Goudet *et al.* 2002; Cano *et al.* 2008). The statistical significance of differences between the sexes in these descriptors was determined using the randomization procedure (10 000 permutations) implemented in the program FSTAT (Goudet 1995). The tests were one-sided, assuming that females were more philopatric. Analyses were conducted using samples from only the downstream portion of the Firetail Creek in which the sexes were identified.

The package HIERFSTAT (Goudet 2005) was used to conduct a nested gene diversity analysis. Significance of *F*-statistics for each hierarchical level was assessed by randomizations. A neighbour-joining tree based on Cavalli-Sforza & Edwards chord distance (1967) was constructed with PHYLIP (Felsenstein 1993). This distance was chosen because it performed best for reconstructing tree topologies in simulated microsatellite data in a study by Takezaki & Nei (1996). Support for tree nodes was found by bootstrapping loci 1000 times. Factorial correspondence analysis (FCA) was performed using the AFC procedure implemented in GENETIX ver. 4.05.2 (Belkhir *et al.* 2004). Assignment of individuals to their populations of origin (combined downstream Firetail Creek sites vs. combined upstream sites) was done in GENECLASS2 (version 2.0.e; Piry *et al.* 2004) with Bayesian criteria.

Results

Genetic diversity

Population genetic diversity indices are shown in Table 1. The total number of alleles per locus ranged from 12 to 41, with a mean of 19.3. Within sites, the mean number of alleles per locus ranged from 1.9 to 15.4, with the lowest numbers (1.9–2.4) occurring in U samples collected above the waterfalls in Firetail Creek. In permutation tests in FSTAT, allelic richness in Firetail Creek was significantly lower ($P < 0.001$) in populations above ($A_R = 2.1$) vs. below ($A_R = 10.8$) waterfalls. Mean expected heterozygosity (H_E) was high and similar at most sites (0.79–0.90), with the exception of U sites where it was much lower (0.14–0.20). This pattern was evident within Firetail Creek, where heterozygosity was significantly lower in populations above (0.17) than below (0.88) the waterfalls ($P < 0.001$).

In a total of 169 tests for each locus and site, significant departures from Hardy–Weinberg equilibrium (HWE) in the direction of heterozygote deficiencies were observed in 22 cases (Table 1). Most of these involved loci *d45* and *d60*, but only four tests remained significant after sequential Bonferroni correction. Global exact probability tests across sites further confirmed departures from HWE at loci *d45* and *d60* ($P < 0.001$) and no departures at the other seven loci. Global tests for HWE across all nine loci were significant in several sites and overall, and again they were caused by *d45* and *d60* (when these loci were excluded from the analysis, no departures from HWE were observed in any site, or overall).

Significant heterozygote deficiencies at loci *d45* and *d60* were not unexpected. In our previous study of paternity (Tatarenkov *et al.* 2008), we deduced the presence of null alleles (at frequencies 0.039 and 0.030 for these two loci) based on the segregation of alleles in progeny from females at sites FT3 and FT5. The presence of null alleles can lead to overestimates in the frequencies of other alleles and potentially influence results of population structure analyses. To prevent such bias, we conducted all analyses twice: using all nine loci, and excluding *d45* and *d60*. In all cases, results were qualitatively identical. Thus, we report results of the full nine-locus analysis, except for the nested genetic analyses when we show both outcomes to demonstrate how close the results were.

Table 1 Genetic variation in populations of *Xiphophorus helleri*

						F_{IS}										
Site	*N*	*A*	A_R	H_E	H_O	9 loci	7 loci	*b69*	*b80*	*d45*	*c45*	*d55*	*d60*	*d33*	*d36*	*d51*
FT	17	12.4	12.2	0.87	0.82	0.06**	0.00	−0.02	−0.17	0.06	0.05	−0.01	**0.42*****	0.08	0.04	0.01
FT0	25	12.8	11.2	0.88	0.87	0.01	−0.03	0.03	−0.06	0.10*	−0.12	−0.01	0.18**	0.00	−0.08	0.03
FT3/4	46	14.8	11.1	0.88	0.87	0.02*	−0.01	−0.05	−0.04	0.09*	−0.02	0.01	0.13***	−0.04	0.10	−0.04
FT5	67	15.4	10.9	0.88	0.87	0.01	−0.01	−0.01	−0.02	0.06	−0.06	−0.01	0.10**	0.01	0.08	−0.03
FT6	16	10.7	10.7	0.88	0.85	0.04*	−0.01	0.11	0.05	0.33**	−0.02	−0.06	0.08	−0.02	−0.12	0.01
FT7	26	11.8	10.3	0.87	0.84	0.04**	0.01	0.04	−0.08	0.16*	−0.02	0.03	0.09**	0.05	0.02	0.03
FT10	29	12.7	10.6	0.87	0.85	0.02	−0.04	−0.01	−0.15	**0.29*****	0.12	−0.11	0.12	−0.07	−0.07	0.06
FT12	22	10.8	10.0	0.87	0.83	0.04	0.02	0.01	0.14	0.16***	−0.06	−0.01	0.05	−0.06	0.00	0.13
FT13	23	11.4	10.4	0.87	0.83	0.04*	−0.02	−0.08	0.12	0.14	−0.15	−0.03	**0.34*****	0.03	−0.01	0.01
U1	28	2.3	2.1	0.14	0.14	0.01	0.08*	NA	NA	−0.20	NA	−0.06	NA	−0.02	−0.06	0.19*
U3	25	2.4	2.3	0.19	0.20	−0.08	−0.11	NA	0.00	0.06	NA	−0.04	NA	−0.05	−0.26	0.00
U4	22	1.9	1.8	0.20	0.22	−0.14	−0.14	NA	NA	−0.14	NA	0.02	NA	−0.36	−0.07	−0.15
C1	21	13.0	11.8	0.88	0.86	0.02	−0.02	−0.10	0.05	0.19*	0.00	0.03	0.10	−0.03	−0.05	−0.04
A	21	12.7	11.7	0.90	0.88	0.03	0.01	−0.02	0.04	0.13*	0.03	0.02	0.05	−0.04	−0.11	0.12
OM	32	11.7	9.7	0.82	0.81	0.01	0.00	0.19***	−0.23	0.06	0.05	−0.01	0.03	0.02	−0.01	0.01
O0	24	10.2	9.2	0.83	0.83	0.01	−0.01	0.13	0.03	0.02	−0.19	−0.01	0.08	0.02	−0.07	−0.02
O1	34	11.3	9.5	0.82	0.78	0.04*	0.01	0.01	0.11	0.11	−0.14	−0.06	**0.17*****	0.01	0.15	−0.04
RM	23	12.1	10.7	0.84	0.82	0.02	−0.02	0.03	−0.06	0.16	−0.22	−0.07	0.20*	0.01	0.01	0.05
R1	21	10.9	10.0	0.83	0.80	0.04	0.02	−0.03	0.01	0.09	0.09	0.04	0.11	−0.01	−0.10	0.17*
R2	20	10.0	9.3	0.79	0.74	0.06*	0.04	0.20**	−0.07	−0.02	0.22	0.08	0.23*	−0.04	0.00	−0.02
Mean	27.1	10.6	9.3	0.76	0.74	**0.03*****	−0.01	0.03	−0.02	**0.11*****	−0.03	−0.01	**0.15*****	−0.01	−0.02	0.03
F_{IT}						0.13	0.10	0.12	0.10	0.18	0.17	0.07	0.26	0.09	0.08	0.09

A, mean number of alleles per locus; A_R, allelic richness; H_E and H_O, expected and observed heterozygosities, respectively; F_{IS}, coefficient of inbreeding shown for individual loci, all 9 loci, and a subset of 7 loci (*d45* and *d60* excluded). Significance of deviations from Hardy–Weinberg equilibrium is indicated by stars (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$), values in bold remained significant after Bonferroni correction.

We found little evidence for genotypic linkage disequilibrium between any pair of loci. Of the 639 pairwise comparisons (after excluding cases where comparisons were not possible to conduct), 29 were significant at the $P < 0.05$ level. These significant cases were randomly distributed among populations and pairs of loci. Only two pairwise comparisons remained significant after correction for multiple testing. The lack of linkage disequilibrium was expected, as microsatellite loci used in our study were from different linkage groups (Walter *et al*. 2004).

Genetic differentiation

Pairwise estimates of population genetic differentiation (F_{ST}) varied considerably using all loci (range 0.00–0.49). F_{ST} values were significant in 128 of the 190 pairwise tests, following sequential Bonferroni correction. The largest genetic differentiation (mean $F_{ST} = 0.39$) was observed in comparisons of the three upstream sites in Firetail Creek vs. all other sites, and all of these pairwise comparisons remained significant after sequential Bonferroni correction. Genetic differentiation between downstream samples was smaller (max $F_{ST} = 0.07$), but 74 comparisons were significant following Bonferroni correction. Most of the significant F_{ST} comparisons were between samples from different creeks, but five were between samples within creeks.

Considering all 20 collection sites, highly significant genetic differentiation (exact probability test, $P < 0.0001$) was observed at each locus, and overall. Genetic differentiation remained highly significant when the divergent upstream samples were excluded from the analysis. Furthermore, significant heterogeneity (Fisher's combined probability test $P < 0.001$) was found within creeks: at five loci in Firetail Creek (excluding upstream populations) and at six loci in Richardson Creek. In Oro Creek, significant heterogeneity was found at locus *c45* ($P = 0.02$) and overall (Fisher's combined probability test, $P = 0.045$).

To evaluate the effect of geographic subdivision at different scales in the absence of obvious physical barriers, we conducted hierarchical analysis using 15 sites from three creeks (excluding the upstream samples from Firetail Creek, as well as sites A and C1). Overall differentiation accounted for 3.0–3.3% of the total variation (depending on whether we used all nine loci or only the seven deduced to carry no null alleles), and

Table 2 Nested genetic analysis of *Xiphophorus helleri* populations. Shown are values of F_{ST} for a given hierarchical level (probabilities in parenthesis). This table shows variation due to geographical separation, without other obvious physical barriers, for a total of 15 sites from three creeks with replicated samples. Upstream samples in Firetail Creek are excluded from the analysis

Hierarchical level	9 loci	7 loci
$F_{Samples/Creek}$	0.008 (0.001)	0.008 (0.001)
$F_{Creeks/Total}$	0.022 (0.001)	0.025 (0.002)
F_{ST}	0.030 (0.001)	0.033 (0.001)

was highly significant (Table 2). Between-site differences within creeks were also significant ($P < 0.001$) and explained 0.8% of overall variation, whereas divergence between creeks explained 2.2–2.5% of the total variation ($P < 0.002$).

To assess the effect of waterfalls on genetic structure, we conducted hierarchical analysis in Firetail Creek, splitting the sites into upstream (above-waterfall) and downstream (below-waterfall) groups (Table 3). The divergence between upstream and downstream sites explained 28–29% of the total variation. Genetic differentiation within each of these two sections of creek was much lower (1%), but nevertheless significant.

The neighbour-joining tree (Fig. 2) revealed several well-supported clusters that reflect geographical separation and isolation by waterfalls. The upstream populations of Firetail Creek are the most distinct, and have strong (100%) bootstrap support. The Oro sites form another strongly supported cluster (bootstrap 100%). The spatially adjacent populations A and C1 join together and are embedded in a cluster formed by the nearby Firetail Creek populations below the waterfalls. This large cluster is separated from the Richardson Creek populations at the 83% level of bootstrapping.

Population differentiation in Firetail Creek (excluding populations above the waterfall) significantly increased with waterway distance ($P < 0.01$, Mantel test; Fig. 3A), but the correlation coefficient was not particularly high (coefficient of determination R^2 explained only 24% of

Table 3 Nested genetic analysis of *Xiphophorus helleri* in Firetail Creek. Shown are values of F_{ST} for a given hierarchical level (probabilities in parenthesis). This table shows variation due to separation from physical barriers, for 12 sites from Firetail Creek divided in two groups: upstream (above the waterfall) and downstream (below the waterfall)

Hierarchical level	9 loci	7 loci
$F_{Samples/Creek\ Section}$	0.010 (0.001)	0.010 (0.001)
$F_{Creek\ Sections/Creek}$	0.291 (0.007)	0.279 (0.003)
$F_{Samples/Creek}$	0.298 (0.001)	0.286 (0.001)

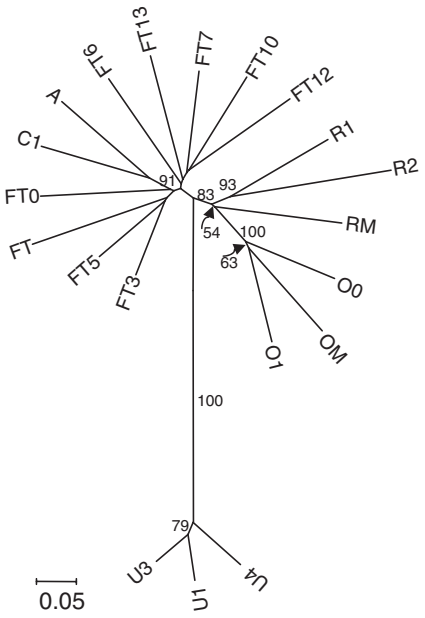

Fig. 2 Neighbour-joining tree of *Xiphophorus helleri* populations based on Cavalli-Sforza & Edwards distance. Values of bootstrap support are shown on the nodes.

the genetic variance). The correlation of genetic (F_{ST}) and geographic distances was higher ($R^2 = 0.448$, Mantel test $P < 0.001$; Fig. 3B) for populations across the full study area (but again excluding the upstream populations). Thus, isolation by distance was apparent at all spatial scales, including the smallest scale of a single creek.

Partial Mantel tests (which included populations above the waterfall) confirmed the important role of waterfalls in structuring genetic variation. When controlling for geographic distance, the correlation of F_{ST} with waterfall presence was significant ($P < 0.001$) in all three tests (see Materials and methods). Interestingly, the highest coefficient of correlation ($R = 0.976$) was obtained in the test that accounted for the first waterfall only, while the correlation was lowest ($R = 0.823$) in the test accounting for each waterfall. This suggests that differentiation between the U sites can largely be explained by isolation by distance. The correlation of genetic (F_{ST}) and geographic distances was significant ($P < 0.05$) when controlling for the first waterfall, as well as for waterfall presence/absence, but not when accounting for the number of waterfalls between sites. Again, this suggests that the largest waterfall played a critical role in subdividing upstream and downstream sites, and that the other waterfalls were less important.

Sex-biased dispersal

Population genetic parameters were estimated separately for males and females from the downstream

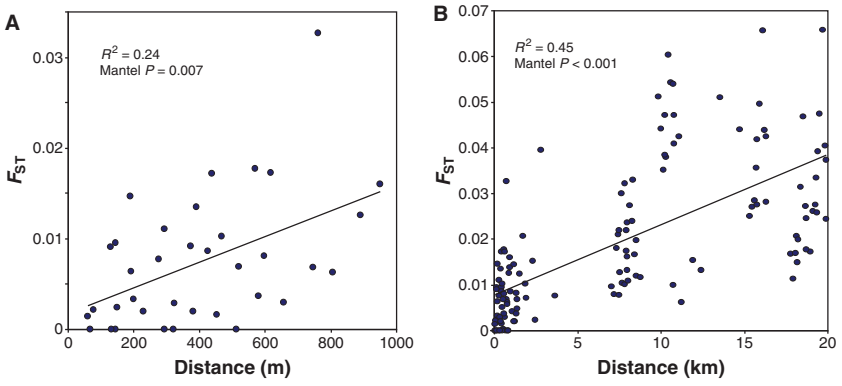

Fig. 3 Isolation by distance (IBD) in *Xiphophorus helleri* populations. A, the IBD pattern in Firetail Creek; B, the IBD correlation in the entire study area.

portion of the Firetail Creek (Table 4). No differences were found between the two sexes at any of the parameters, thus lending no support for the hypothesis that males dispersed more than females.

Recaptures of tagged males

A total of 158 males were captured in Firetail Creek at the beginning and end of the dry season. Twenty-five males (16% of the total, or 28% of the December sample, $N = 88$) were captured during both periods. The 70 males newly caught in April and May might include recently matured juveniles in addition to adults that were present but not captured in December.

Of the 25 males captured during both seasons, 18 were caught at the same site. Of the seven males that had dispersed, five moved to adjacent pools. These movements occurred in both upstream and downstream directions and covered distances of 20–50 m. Two males moved longer distances: male FTa01 moved about 120 m from site FT0 into FT3/4 (and sired some progeny with two females there), and male FTa15 moved about 300 m from site FT2 into FT7 (without leaving any progeny in either FT3/4 or FT5; Tatarenkov *et al.* 2008). Besides these instances of movement based on physical tags, we also found several cases of dispersal based on our genetic analyses (as discussed below).

Table 4 Gene diversity, *F*-statistics, mean assignment index (*mAIc*) and variance of assignment index (*vAIc*) for each sex of *Xiphophorus helleri* in downstream Firetail Creek. *P* is the probability that these statistics differ significantly between the two sexes (one-sided test)

Sex	H_S	F_{ST}	F_{IS}	*mAIc*	*vAIc*
Males	0.871	0.007	0.031	0.332	10.184
Females	0.880	0.007	0.014	−0.245	12.529
P	0.90	0.52	0.19	0.91	0.88

Movement across waterfalls

With 100% probability, GENECLASS2 assigned two individuals (both males) from the lower portion of Firetail Creek to the upstream (U) population. At seven loci, these males were homozygous for main alleles (those present in highest frequency) at U sites, and at two other loci they were heterozygous for such alleles. These males were captured in December 2006 and they may have been carried downstream across waterfalls to the lower part of the creek during the preceding rainy season. One was found at site FT13, about 50 m from the nearest waterfall, and the other at site FT7, about 350 m from the waterfall. Furthermore, in April at site FT9, we captured one male who by similar reasoning was an apparent F1 progeny from a cross between a local swordtail and a fish from an upstream population (Fig. 4).

Discussion

Within-creek dispersal

Our tagging experiment documented considerable movement of green swordtail males between pools in Firetail Creek. Seven of 25 recaptured individuals (28%) changed their pools of residence during 4 months. Most relocations were to adjacent pools, but two males moved longer distances (up to 300 m) and traversed multiple pools. These are minimum dispersal estimates, because some males who may have moved to more distant sites would not have been recaptured and movements in which males returned to their initial pool after visiting other sites would have gone unnoticed.

Even though we did not mark females directly, dispersal is by no means restricted to males. In our parentage analysis (Tatarenkov *et al.* 2008), some plausible movements of females were inferred (but not discussed in that study). For example, female FT5-40, captured in

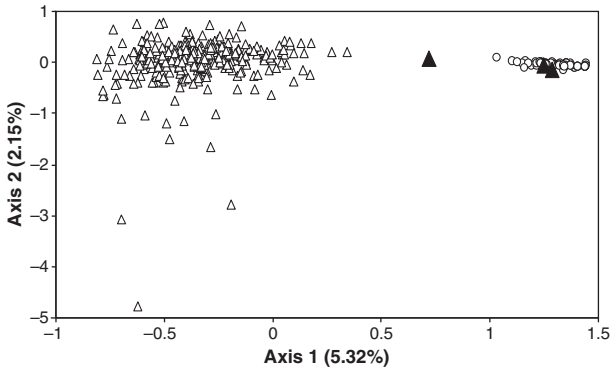

Fig. 4 Results of factorial correspondence analysis (FCA) based on individual genotypes. Included in the plot are individuals collected in the upstream or above-waterfall sites (circles) and in the downstream or below-waterfall sites (triangles) of Firetail Creek. Two enlarged filled triangles embedded among circles depict two individuals that migrated downstream across waterfalls. Another enlarged triangle roughly midway between the two groups corresponds to an offspring from a cross between an individual from an upstream population and an individual from the downstream portion of Firetail Creek.

May in site FT5, produced progeny fathered by a male who was captured in site FT1 in December and in April (and thus was likely to reside permanently in FT1 during whole studied period). The parsimonious explanation is that the female moved from pool FT1 to pool FT5 after mating with that male. Another female (FT5-15) from site FT5 had progeny from a male of site FT3/4 (where he was captured in December and April and sired progeny with other females from that pool). Altogether, there were five females for which past movements of this sort were firmly established from the evidence of genetic parentage. Because *Xiphophorus* females are able to store viable sperm, it is impossible to say precisely when the movements took place, but they probably occurred within a few months prior (Constantz 1989; Potter & Kramer 2000). Additional cases similarly indicated movement between pools, but it was not possible to say with certainty whether such dispersal was by males or females.

As only males were tagged, it was not possible to evaluate from direct observations whether a sexual bias in dispersal exists. However, no sex-biased dispersal was detected using tests that compare population genetic parameters calculated separately for males and females (Goudet *et al.* 2002). Note, however, that such tests may not be powerful in species with overlapping generations; but, on the other hand, if migrants do not reproduce in the new population, the chances for detecting sex-biased dispersal may improve (Cano *et al.* 2008).

Microgeographic differentiation

Despite the relatively high incidence of *Xiphophorus* movements within Firetail Creek, we found significant genetic differentiation between pools within creeks, even in the absence of obvious physical barriers such as waterfalls.

How well does the observed number of tagged dispersed individuals fit with the pattern of genetic differentiation? Under the island model, the number of migrants per generation is estimated as $N_m = (1-F_{ST})/4\,F_{ST}$ (Wright 1969). F_{ST} in the downstream populations is about 3% (Table 2), giving the estimated number of effective migrants per generation $N_m \sim 8.1$. The private allele method (Barton & Slatkin 1986; as implemented in Genepop version 4.0), also under the island model, produced a similar estimate: $N_m = 8.2$. Under the isolation by distance model, which fits our case, the number of migrants necessary to attain such an F_{ST} value is expected to be even higher, as many of the movements would occur between neighbouring sites that are genetically similar, and thus making each act of dispersal relatively less important in erasing genetic differences. Using physical tagging we observed that seven males moved between pools within one creek during a period of 4 months. Assuming that females disperse as well as males, and taking into account a female-biased sex ratio (2:1) typical of swordtails (Franck *et al.* 1998), our genetic and direct estimates of dispersal agree that the number of immigrants into a pool is at least several individuals per generation. It should be noted that a direct observation of dispersal is not necessarily tantamount to gene flow, because immigrant individuals may fail to reproduce in the new settings (Whitlock & McCauley 1999). This may be particularly relevant in the case of *X. helleri* males who are under strong sexual selection. However, our data did show that at least some males who dispersed were reproductively successful. Furthermore, gravid females, who often carry progeny and sperm from more than one male, should be very effective in generating gene flow. Our genetic data did not detect any bias in dispersal rates between males and females. Taken altogether, the evidence indicates that genetic differentiation between neighbouring sites of a creek exists despite high gene flow.

Microgeographic differentiation on similar spatial scales has been reported previously for other stream fishes (White & Turner 1984; Congdon 1995), with genetic drift being the suspected culprit. Stochastic fluctuations of allele frequencies also seem likely to explain differentiation among the swordtail demes in Firetail Creek. Each pool typically harbours only a small number of individuals at any one time. For example, during exhaustive searches in the two largest pools, we cap-

tured 29 females in site FT3/4 and 42 females in FT5. The number of males is even lower; a rather intensive search in December 2006 resulted in only 88 males captured along the lower 1 km of Firetail Creek. Such small sizes of local demes can result in high allele-frequency variances between successive generations (Hansen *et al.* 2002; Fraser *et al.* 2004, 2007; Jensen *et al.* 2005b).

Genetic drift will be further amplified by the mating system in *Xiphophorus*, in which a few males may sire most of the progeny (Tatarenkov *et al.* 2008). For example, we found that a single most successful male sired almost 29% of all progeny in pool FT5, even though 35 males took part in reproduction there. Such reproductive skew further decreases effective population size and increases the potential role of random drift. Skew in reproductive success, combined with sex ratio bias typical for *Xiphophorus* should yield effective population sizes that are considerably smaller than census sizes (Hedrick 2005). High reproductive skew, although not necessarily caused by sexual selection as in *X. helleri*, has been implicated in creating genetic patchiness and temporal variation in many marine invertebrates with high potential for dispersal (Hedgecock 1994a,b).

In addition to small effective population size and associated genetic drift in pools along a creek, genetic differentiation may arise as a result of local extinction/recolonization dynamics because some pools dry out completely during dry seasons, and their colonization by a limited number of individuals can result in genetic differentiation due to founder effect (Vrijenhoek 1979; Vrijenhoek & Lerman 1982; Barr *et al.* 2008 and references therein). However, whereas extinction/recolonization might in principle play a significant role in shaping population structure of *X. helleri*, it seems that its impact in Firetail Creek was largely erased by frequent dispersal between adjacent pools. Typically, perturbed systems are characterized by haphazard genetic differentiation, but we observed an isolation by distance (IBD) pattern in the lower part of Firetail Creek.

Differentiation between creeks

Not surprisingly, the between-creek variance was the largest component of genetic differentiation in the current study, accounting for about 75% of the total genetic heterogeneity due to geographic separation (Table 2), or about 2.5% of the total variation. Although waterway distances between creeks were 20 km at most, such geographic separation, even in the absence of impenetrable physical barriers to movement, must be a substantial dispersal impediment for small fish such as swordtails (mature fish range from 22–78 mm in the Bladen Branch River population, Meyer 2006). Other small livebearing fishes surveyed across similar geographical scales have shown even higher differentiation. For example, the mosquitofish *Poecilia holbrooki* displayed $F_{ST} = 0.071$ among small tributaries of the Savannah River (Smith *et al.* 1989); and the guppy *Poecilia reticulata* showed $F_{ST} = 0.114$ within few-kilometer stretches, separated by high waterfalls, of a small river (Crispo *et al.* 2006). By contrast, F_{ST} values similar to those in the current swordtail survey (<3%) have been associated with much larger geographic scales (tens to hundreds or thousands km) in some bigger and more mobile fish such as salmon (see Appendix 2 in Hendry & Stearns 2004; Primmer *et al.* 2006).

Waterfalls and population structure

The genetic heterogeneity uncovered between nearby pools of *X. helleri* demonstrates the power of genetic drift to produce genetic differentiation even in the presence of appreciable gene flow. Such power becomes even more pronounced for populations separated by waterfalls. The three sites above waterfalls were drastically different (minimum $F_{ST} = 0.30$) from sites just below waterfalls in Firetail Creek (Table 3). Indeed, this differentiation far exceeded the F_{ST} values (maximum 0.07) observed between creeks up to 20 km apart but not separated by waterfalls. The populations above waterfalls also displayed greatly decreased variation: only 2.1 alleles per locus and mean heterozygosity values of $H_E = 0.17$ (vs. 10.8 alleles per locus and $H_E = 0.88$ just below waterfalls). Furthermore, three loci that each harboured between 12 and 16 alleles at the downstream sites in Firetail Creek were each fixed for one allele in the upstream demes. Altogether, our data thus show that upstream populations experienced a drastic reduction of diversity, most likely due to founder effect.

Interestingly, even though all three upstream populations in Firetail Creek are themselves separated from each other by waterfall(s), their genetic compositions were similar. Most likely, portions of Firetail Creek above the waterfalls were populated during one of the major floods that occur occasionally in the region (e.g. Vrijenhoek & Lerman 1982). Another possibility is that the U sites were founded by incidental transfers by predators such as birds. In any case, such events must be rare, because we did not find any *Xiphophorus* above waterfalls in either Oro or Richardson Creeks, although the habitat seemed suitable. Considering that the uppermost upstream populations (U3 and U4) are not genetically depauperate compared to the lower-positioned deme (U1), it seems likely that the founders initially arrived in the upper reaches of the Firetail Creek, from whence their descendants migrated downstream. Such a hypothesis also meshes well with results of the partial

Mantel tests in explaining why we did not see more structuring effects of waterfalls beyond the first and highest.

The very distinct genetic composition of the upstream populations makes it easy to reliably detect potential downstream migration across waterfalls. In our sample of 298 adults from the lower portion of Firetail Creek, two individuals were identified as migrants from populations above waterfalls, and another was a first-generation offspring of a cross between a local swordtail and one that came from an upstream deme (Fig. 4). Although downstream migration across waterfalls does take place, it seems not to be substantial enough to noticeably change genetic composition in the lower stretches of Firetail Creek. Thus, it was not the case that sites closest to waterfalls (e.g. FT12, FT13) had an increased frequency of alleles typical for upstream populations, compared to sites closer to the stream mouth (e.g. FT0, FT3), nor did we see that alleles predominant in the upstream demes had an increased frequency in Firetail Creek when compared to Oro and Richardson Creeks.

Waterfalls have been found to be a formidable obstacle to fish dispersal in several studies (e.g. Ryman & Ståhl 1981; Taylor *et al.* 2003b; Poissant *et al.* 2005), including other poeciliids (Vrijenhoek 1979; Shaw *et al.* 1994; Crispo *et al.* 2006). The analysis by Crispo *et al.* (2006) is particularly relevant to our study due to similarities in spatial scale and habitat. In that study, waterfalls higher than two meters partitioned demes in ways that explained 23% of the total genetic variation (similar to 29% in our study). More generally, populations in upstream reaches of rivers often exhibit lower genetic variation (Shaw *et al.* 1994), especially when isolated by waterfalls. However, *Xiphophorus* may be extreme in this respect; we observed a five-fold to six-fold drop in heterozygosity and allelic richness between neighbouring samples separated by a waterfall. While occasional downstream movement across a waterfall (as documented by two cases in our study) may inhibit further divergence between upper and lower sections of a creek, it alone is of no help in restoring genetic variation to upstream demes.

Summary

Overall, the population structure of the green swordtail appears to be similar to other poecilliids (Vrijenhoek 1979; Smith *et al.*1989; Shaw *et al.* 1994; Congdon 1995; Crispo *et al.* 2006). At the microgeographic level of a creek, demes inhabiting different pools are differentiated despite high dispersal. Such heterogeneity is mostly a result of random changes due to genetic drift, because these subpopulations are small and further impacted by the highly skewed reproductive output of males. Although local demes are prone to extinction and recolonization, the IBD along Firetial Creek indicates at least a quasi-equilibrium between genetic drift and migration, a sign that this creek has not experienced major perturbations recently. Occasional severe drought might decimate local sites that later are recolonized from nearby pools or from the river, thus producing a meta-population. Over a larger spatial scale, populations also follow an IBD pattern, basically indicating a quasi-equilibrium among mutation, migration, and genetic drift. This pattern of slowly accumulating differences is complicated by the presence of waterfall barriers, which can maintain substantial differentiation over short distances.

The results of our study should have relevance to other fishes of similar size and biology. They suggest that isolated populations, such as those locked above waterfalls, have a good potential to develop local adaptations, as well as to diverge overall from downstream populations. But our results also suggest that some obstacles apply to such outcomes. First, isolated populations may show a paucity of genetic polymorphism, thus requiring that adaptive characters sometimes may have to await *de novo* mutations rather than rely on standing genetic variation alone. Second, for the local adaptations to spread, selection must be rather strong to overcome the pronounced effects of genetic drift. Downstream populations, on the other hand, represent a system of more highly interconnected small demes whose genetic composition varies through time, one implication being that local selection sometimes can act on a whole-creek (or larger) scale.

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